



### REMARKS

Claim 2-60 having been canceled, claim 1 having been amended, and claims 61-119 having been added, the currently pending claims are claims 1 and 61-119.

The present application is a U.S. national stage application of PCT US99 23731. In the international application, claim 1 was amended under Article 34 PCT to incorporate the limitations of claim 2 as originally filed, and the claims were renumbered to reflect this change, such that claims 1-60 were pending in the international application upon entry into the national stage. The International Preliminary Examination Report was issued on the amended international claim set, claims 1-60. However, Applicant would like to have the Article 34 amendment "reversed" for purposes of the U.S. examination, such that claim 1 as originally filed in the international application is fully examined. There are also certain other claim amendments Applicant would like to present at this time.

Accordingly, claim 1 is amended herewith to restore it to its original form as originally filed in the international application. In order to effect this amendment, it is necessary to cancel claims 2-60 in the present application and replace them with a new claim set beginning with claim 61. New claims 61-88 thus correspond directly to claims 2-29 as originally filed in the international application (except that dependencies have been changed, as required); new claim 89 corresponds to originally filed claim 31 except that it is rewritten in independent form and includes the proviso that "when the first stabilizing group is Pro-, the second stabilizing group is not -Pro-Xaa"; and new claims 90-119 correspond to claims 32-61 as originally filed in the international application. There is no new claim that corresponds to claim 30 as originally filed in the international application.

**Preliminary Amendment**

Applicant(s): Elliot ALTMAN

Serial No.: 09 701,947

Filed: 5 December 2000

For: STABILIZED BIOACTIVE PEPTIDES AND METHODS OF IDENTIFICATION, SYNTHESIS AND USE

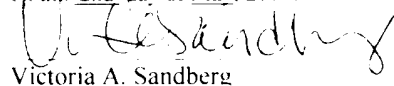
Page 11 of 11

**Conclusion**

The Examiner is invited to contact Applicant's Representatives, at the below-listed telephone number, if there are any questions regarding this Preliminary Amendment or if prosecution of this application may be assisted thereby.

**CERTIFICATE UNDER 37 C.F.R. § 1.8**

The undersigned hereby certifies that this paper is being deposited in the United States Postal Service, as first class mail, in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on this 2nd day of May, 2001.

  
Victoria A. Sandberg

Respectfully submitted,

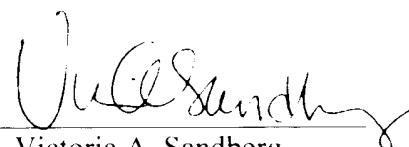
Elliot ALTMAN

By his Representatives,  
Mueiting, Raasch & Gebhardt, P.A.  
P.O. Box 581415  
Minneapolis, MN 55458-1415  
Phone: (612) 305-1220  
Facsimile: (612) 305-1228  
**Customer Number 26813**

May 2, 2001

Date

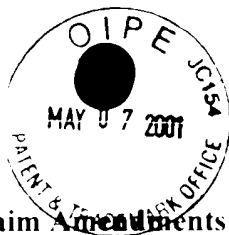
By:



Victoria A. Sandberg

Reg. No. 41,287

Direct Dial (612) 305-1226



Appendix A  
Claim Amendments with Notations To Indicate Changes Made  
Serial No.: 09/701,947

Filed: 5 December 2000

For: STABILIZED BIOACTIVE PEPTIDES AND METHODS OF  
IDENTIFICATION, SYNTHESIS AND USE

---

In the Claims

1. **(Amended)** A recombinant method for identifying a bioactive peptide comprising:

(a) transforming a host cell [comprising Lac repressor protein] with an expression vector comprising a tightly regulable control region operably linked to a nucleic acid sequence encoding a peptide], wherein the tightly regulable control region of the expression vector comprises at least a portion of the wild-type *E. coli lac* promoter/operator region, said portion comprising auxiliary *lac* operator O3, a CAP binding region, the -35 *lac* promoter site, the -10 *lac* promoter site, *lac* operator O1, *lacZ* Shine-Dalgarno sequence and a spacer region];

(b) growing the transformed cell under conditions that repress expression of the peptide;

(c) inducing expression of the peptide in the transformed host cell; and

(d) determining whether expression of the peptide is inhibitory of host cell growth, wherein inhibition of host cell growth is indicative of the expression of a bioactive peptide.

61. **(New)** The method of claim 1 wherein the tightly regulable control region of the expression vector comprises at least a portion of the wild-type *E. coli lac* promoter/operator region, said portion comprising auxiliary *lac* operator O3, a CAP binding region, the -35 *lac* promoter site, the -10 *lac* promoter site, *lac* operator O1, *lacZ* Shine-Dalgarno sequence and a spacer region; and wherein the transformed host cell comprises an amount of Lac repressor protein effective to repress expression of the peptide during step (b).

62. **(New)** The method of claim 61 wherein the host cell is a bacterium.

63. (New) The method of claim 62 wherein the bacterium is a gram positive bacterium.

64. (New) The method of claim 62 wherein the bacterium is gram negative bacterium.

65. (New) The method of claim 62 wherein the bacterium is *E. coli*.

66. (New) The method of claim 61 wherein the host cell is a microbial pathogen.

67. (New) The method of claim 66 wherein the microbial pathogen is a member of a genus selected from the group consisting of *Streptococcus*, *Staphylococcus* and *Enterococcus*.

68. (New) The method of claim 61 wherein the expression vector comprising the nucleic acid sequence encoding the peptide is a first expression vector, and wherein the host cell is further transformed, prior to step (b), with a second expression vector comprising a promoter operably linked to a gene encoding a Lac repressor protein.

69. (New) The method of claim 61 wherein the expression vector has the identifying characteristics of pLAC11 (ATCC No. 207108).

70. (New) The method of claim 69 wherein the expression vector is pLAC11 (ATCC No. 207108).

71. (New) The method of claim 1 wherein the host cell comprises proteases or peptidases or both.

72. (New) The method of claim 1 wherein the host cell has not been modified to reduce or eliminate the expression of naturally expressed proteases or peptidases.

73. (New) The method of claim 1 wherein the host cell is a prokaryote.

74. (New) The method of claim 1 wherein the host cell is a microbial pathogen.

75. (New) The method of claim 74 wherein the microbial pathogen is a member of a genus selected from the group consisting of *Streptococcus*, *Staphylococcus* and *Enterococcus*.

76. (New) The method of claim 1 wherein the host cell is a eukaryotic cell.

77. (New) The method of claim 76 wherein the eukaryotic cell is a mammalian cell.

78. (New) The method of claim 76 wherein the eukaryotic cell is a cancer cell.

79. (New) The method of claim 1 wherein the host cell is a protozoan.

80. (New) The method of claim 1 wherein the peptide comprises a first stabilizing group comprising the N-terminus of the peptide and a second stabilizing group comprising the C-terminus of the peptide.

81. (New) The method of claim 80 wherein the first stabilizing group is selected from the group consisting of a small stable protein, Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro-; and wherein the second stabilizing group is selected from the group consisting of a small stable protein, -Pro-, -Pro-Pro-, -Pro-Xaa and -Pro-Pro-Xaa.

82. (New) The method of claim 81 wherein the small stable protein is selected from the group consisting of Rop protein, glutathione sulfotransferase, thioredoxin, maltose binding protein and glutathione reductase.

83. (New) The method of claim 1 wherein the peptide comprises a stabilizing motif.

84. (New) The method of claim 83 wherein the stabilizing motif comprises a hydrophilic  $\alpha$ -helix motif.

85. (New) The method of claim 83 wherein the stabilizing motif comprises an opposite charge ending motif.

86. (New) The method of claim 1 wherein the peptide comprises a randomized amino acid sequence.

87. (New) The method of claim 86 wherein the peptide comprises a first stabilizing group comprising the N-terminus of the peptide and a second stabilizing group comprising the C-terminus of the peptide.

88. (New) The method of claim 86 wherein the peptide comprises a stabilizing motif.

89. (New) A bioactive peptide comprising a first stabilizing group comprising the N-terminus of the bioactive peptide and a second stabilizing group comprising the C-terminus of the bioactive peptide, wherein the first stabilizing group is selected from the group consisting of a small stable protein, Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro-, and wherein the second stabilizing group is selected from the group consisting of a small stable protein, -Pro-, -Pro-Pro-, -Pro-Xaa and -Pro-Pro-Xaa, with the proviso that when the first stabilizing group is Pro-, the second stabilizing group is not -Pro-Xaa.

90. (New) The bioactive peptide of claim 89 wherein the small stable protein is selected from the group consisting of Rop protein, glutathione sulfotransferase, thioredoxin, maltose binding protein, and glutathione reductase.

91. (New) The bioactive peptide of claim 89 wherein the first stabilizing group is Pro-Pro- and the second stabilizing group is -Pro-Pro.

92. (New) The bioactive peptide of claim 89 wherein at least one of the first and second stabilizing groups comprises a small stable protein.

93. (New) The bioactive peptide of claim 92 wherein the small stable protein is a four-helix bundle protein.

94. (New) The bioactive peptide of claim 92 wherein the small stable protein is selected from the group consisting of Rop protein, glutathione sulfotransferase, thioredoxin, maltose binding protein and glutathione reductase.

95. (New) The bioactive peptide of claim 94 wherein the small stable protein is Rop protein.

96. (New) The bioactive peptide of claim 89 which is an antimicrobial peptide.

97. (New) The bioactive peptide of claim 89 which is a therapeutic peptide drug.

98. (New) A bioactive peptide comprising a plurality of sequential uniformly charged amino acids comprising the N-terminus of the bioactive peptide and a plurality of sequential oppositely charged amino acids comprising the C-terminus of the bioactive peptide.

99. (New) A fusion protein comprising a four-helix bundle protein and a polypeptide.

100. (New) The fusion protein of claim 99 wherein the four-helix bundle protein is Rop protein.

101. (New) The fusion protein of claim 100 wherein the polypeptide comprises a bioactive peptide.

102. (New) The fusion protein of claim 100 wherein the four-helix bundle protein is covalently linked at its C-terminus to the N-terminus of the polypeptide.

103. (New) The fusion protein of claim 100 wherein the four-helix bundle protein is covalently linked at its N-terminus to the C-terminus of the polypeptide.

104. (New) A polypeptide comprising:

a bioactive peptide comprising (a) a first stabilizing group selected from the group consisting of a small stable protein, -Pro-, -Pro-Pro-, -Xaa-Pro- and -Xaa-Pro-Pro- and (b) a second stabilizing group selected from the group consisting of a small stable protein, -Pro-, -Pro-Pro-, -Pro-Xaa and -Pro-Pro-Xaa; and

a cleavage site immediately preceding the first stabilizing group;  
wherein the second stabilizing group comprises the C-terminus of the polypeptide.

105. (New) A polypeptide comprising:

a bioactive peptide comprising (a) a first stabilizing group selected from the group consisting of Pro, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro- and (b) a second stabilizing group selected from the group consisting of -Pro-, -Pro-Pro-, -Pro-Xaa- and -Pro-Pro-Xaa-; and

a cleavage site immediately following the second stabilizing group;  
wherein the first stabilizing group comprises the N-terminus of the polypeptide.

106. (New) A polypeptide comprising:

a bioactive peptide comprising a plurality of sequential uniformly charged amino acids comprising the N-terminus of the bioactive peptide and a plurality of sequential oppositely charged amino acids comprising the C-terminus of the bioactive peptide; and



a cleavage site immediately preceding the plurality of sequential uniformly charged amino acids.

107. (New) A polypeptide comprising:

a bioactive peptide comprising a plurality of sequential uniformly charged amino acids comprising the N-terminus of the bioactive peptide and a plurality of sequential oppositely charged amino acids comprising the C-terminus of the bioactive peptide; and

a cleavage site immediately following the plurality of sequential oppositely charged amino acids.

108. (New) A method for using an antimicrobial peptide comprising:

covalently linking a first stabilizing group to the N-terminus of the antimicrobial peptide and a second stabilizing group to the C-terminus of the antimicrobial peptide to yield a stabilized antimicrobial peptide; and

contacting a microbe with the stabilized antimicrobial peptide.

109. (New) The method of claim 108 wherein the first stabilizing group is selected from the group consisting of a small stable protein, Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro-; and wherein the second stabilizing group is selected from the group consisting of a small stable protein, -Pro, -Pro-Pro, -Pro-Xaa and -Pro-Pro-Xaa.

110. (New) The method of claim 108 wherein the first stabilizing group is selected from the group consisting of Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro- and the second stabilizing group is selected from the group consisting of -Pro, -Pro-Pro, -Pro-Xaa and Pro-Pro-Xaa.

111. (New) A method for using an antimicrobial peptide comprising:

covalently linking a plurality of sequential uniformly charged amino acids to the N-terminus of the antimicrobial peptide and covalently linking a plurality of sequential

oppositely charged amino acids to the C-terminus of the antimicrobial peptide to yield a stabilized antimicrobial peptide; and

contacting a microbe with the stabilized antimicrobial peptide.

112. (New) A method for treating a patient having a condition treatable with a peptide drug comprising administering to the patient a stabilized form of the peptide drug.

113. (New) The method of claim 112 wherein the stabilized form of the peptide drug comprises a first stabilizing group comprising the N-terminus of the peptide drug and a second stabilizing group comprising the C-terminus of the peptide drug.

114. (New) The method of claim 113 wherein the first stabilizing group is selected from the group consisting of a small stable protein, Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro-; and wherein the second stabilizing group is selected from the group consisting of a small stable protein, -Pro, -Pro-Pro, -Pro-Xaa and -Pro-Pro-Xaa.

115. (New) The method of claim 114 wherein the small stable protein is a four-helix bundle protein.

116. (New) The method of claim 114 wherein the small stable protein is selected from the group consisting of Rop protein, glutathione sulfotransferase, thioredoxin, maltose binding protein and glutathione reductase.

117. (New) The method of claim 113 further comprising, prior to administration of the stabilized form of the peptide drug, covalently linking the first stabilizing group to the N-terminus of a peptide drug and covalently linking the second stabilizing group to the C-terminus of the peptide drug to yield the stabilized form of the peptide drug.

118. (New) The method of claim 112 wherein the stabilized form of the peptide drug comprises an opposite charge ending motif.

119. (New) The method of claim 118 further comprising, prior to administration of the stabilized form of the peptide drug, covalently linking a plurality of sequential uniformly charged amino acids to the N-terminus of the peptide drug and covalently linking a plurality of sequential oppositely charged amino acids comprising the C-terminus of the peptide drug to yield the stabilized form of the peptide drug.